# DNA Looping Mediated Transcriptional Regulation

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**A thesis submitted for the degree of Doctor of Philosophy**

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# Contents

# Chapter 8: References for chapters 1 and 6

# Abstract

Protein binding to DNA sequences is the foundation of transcriptional regulation. By binding at specific DNA sequences, such as promoters, proteins can recruit other proteins to regulate gene transcription. Some proteins, which bind at separated DNA binding sites, can interact via the formation of a DNA loop. DNA looping is essential in many processes, such as replication, recombination and gene regulation. In prokaryotic systems, DNA looping is involved in some genetic switches, which can control bacteriophage lysogenic/lytic pathways or bacterial catabolic pathways. Small DNA loops are essential for effective repression of several operons in bacteria. In eukaryotic systems, enhancers are distal gene regulatory elements, which can be located far away from the promoter. The formation of DNA looping is thought to be necessary for the function of enhancers.

Bacteriophage λ CI repressor activates the transcription of its own gene, while the CI mediated looping represses its own transcription. This DNA looping mediated long-range repression improves the efficiency of the lambda lysogenic/lytic switch. However, evidence in the literature suggests an additional activation effect of λ CI DNA looping. In Chapters 2 and 3, I investigated this long-range λ CI DNA looping mediated transcriptional activation. By using a synthetic λ CI DNA looping reporter system, I confirmed that λ CI DNA looping can mediate enhancer-like long-range transcriptional activation. In vivo experiments showed that the λ PRM promoter was activated by the α C-terminal domain (CTD) of RNA polymerase contacting an UP element located 2.3 kilobases away from the PRM promoter. A physicochemical model of the in vivo data showed that an RNA polymerase α subunit recruitment mechanism could fully explain this activation effect. DNA-protein structural modelling found that the bending of linker sequence between OL2 and the UP element is required for the contact.

The efficiency of long range DNA looping has been studied in Chapter 4. In vivo Lac looping and lambda CI DNA looping constructs were used to generate data for calculating DNA looping efficiency. DNA loop sizes ranging

from 250 bp to 10000 bp were tested. Tethered particle motion (TPM) experiments, performed by our collaborators, generated in vitro DNA looping data by using Lac mediated DNA loops ranging from 600 bp to 3200 bp. Based on these in vitro and in vitro data, mathematical modelling calculated DNA looping parameters for understanding DNA looping efficiency.

The insertion of DNA looping constructs into the *E.coli* chromosome (by using a bacteriophage integrase based approach) also led us to make a one-step integration system (OSIP), described in Chapter 5. The OSIP system is a set of OSIP plasmids, which can mediate one-step bacterial chromosomal integration of DNA sequences. The cloning module of each OSIP plasmid has both the integrase gene and corresponding att sequences, which are required for integrating the OSIP plasmid into the bacterial chromosome. An integration protocol, called clonetegration, was developed by coupling the OSIP system with in vitro isothermal DNA assembly. Clonetegration bypasses plasmid propagation and purification procedures by transferring assembly products directly into target competent cells.

# Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to LUN CUI and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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# List of Publications

1. **Cui, L.**[1], Murchland, I.[1], Shearwin, K. E., and Dodd, I. B. (2013) Enhancer-like long-range transcriptional activation by λ CI-mediated DNA looping. *Proc Natl Acad Sci U S A* 110, 2922-2927.
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2. **Cui, L.**, Murchland, I., Dodd, I. B., Shearwin, K. E. (2013) Bacteriophage lambda repressor mediates the formation of a complex enhancer-like structure. *Transcription*, 4(5).
   Copyright © 2013 Landes Bioscience
3. Priest, D. G., **Cui, L.**, Kumar, S., Dunlap, D., Dodd, I. B., and Shearwin, K. E. (2013) Quantitation of the DNA tethering effect in long-range DNA looping in vivo and in vitro using the Lac and λ repressors. *Proc Natl Acad Sci U S A*, doi:10.1073/pnas.1317817111. (Online Early Edition)
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4. St-Pierre, F.[⊥], **Cui, L.**[⊥], Priest, D. G., Endy, D., Dodd, I. B., and Shearwin, K. E. (2013) One-Step Cloning and Chromosomal Integration of DNA. *ACS Synthetic Biology,* 2 537-541.
   Copyright © 2013 American Chemical Society
5. **Cui, L.**[‡], St-Pierre, F.[‡] and Shearwin, K. E. (2013) Repurposing site-specific recombinases for synthetic biology. *Future Microbiology*, 8(11) 1361-1364□
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